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## Summary

Magnetic resonance imaging (MRI) was used to compare the effect of two modes of lower-extremity exercise training on the mass (volume) of posterior leg group (PLG) muscles (soleus, flexor hallucis longus, tibialis posterior, lateral and medial gastrocnemius, and flexor digitorum longus) on 19 men (ages 32–42 years) subjected to intense dynamic–isotonic (ITE, cycle ergometer, number of subjects (N) = 7), isokinetic (IKE, torque ergometer, N = 7), and no exercise (NOE, N = 5) training for 60 min/day during head-down bed rest (HDBR). Total volume of the PLG muscles decreased ( $p < 0.05$ ) similarly: ITE =  $4.3 \pm \text{SE } 1.6\%$ , IKE =  $7.7 \pm 1.6\%$ , and NOE =  $6.3 \pm 0.8\%$ ; combined volume (N = 19) loss was  $6.1 \pm 0.9\%$ . Ranges of volume changes were 2.6% to –9.0% (ITE), –2.1 to –14.9% (IKE), and –3.4% to –8.1% (NOE). Correlation coefficients (r) of muscle volume versus thickness, measured with ultrasonography, were: ITE  $r = 0.79$  ( $p < 0.05$ ), IKE  $r = 0.27$  (not significant (NS)), and NOE  $r = 0.63$  (NS). Leg-muscle volume and thickness were highly correlated ( $r = 0.79$ ) when plasma volume was maintained during HDBR with ITE. Thus, neither intensive lower extremity ITE nor IKE training influence the normal non-exercised posterior leg muscle atrophy during HDBR. The relationship of muscle volume and thickness may depend on the mode of exercise training associated with the maintenance of plasma volume.

## Introduction

Skeletal muscle atrophy, defined as a “decrease in size of a part or tissue after full development has been attained: a wasting away of tissue” (ref. 1), occurs with disuse and especially during prolonged bed rest (refs. 2–10) and spaceflight (refs. 7 and 11). The ever present question is whether to maintain astronauts’ physical capabilities at eugravity (Earth gravity) levels during spaceflight, or to allow for some deconditioning and restore the capabilities prior to reentry. The answer depends on the type and length of the flight. With 30-day 6-deg HDBR, aerobic work capacity ( $\dot{V}O_2$  peak) can be maintained at pre-bed-rest levels utilizing intense, intermittent cycle ergometer

exercise training (ref. 12). Thigh muscle total work and peak torque can also be maintained or increased with specific isokinetic exercise training (ref. 13). Changes in active and passive lower extremity muscle thicknesses indirectly indicate the effect of general isotonic and isokinetic training on specific muscle anatomy (ref. 14). Posterior leg-muscle volumes were measured using MRI in this study.

The purpose for using MRI in this phase of the study was to determine the effect of intense ITE and IKE training during HDBR on posterior leg-muscle volumes, and to compare the MRI measurements with comparable leg muscle thicknesses measured by ultrasonography.

The authors express their warm gratitude and appreciation to the 125 people who had a significant role in this study.

## Methods

The subjects were 19 men ( $36 \pm \text{SD } 4$  yr,  $178 \pm 7$  cm ht,  $76.5 \pm 7.6$  kg,  $1.94 \pm 0.12$  m<sup>2</sup>,  $44.2 \pm 7.7$  ml O<sub>2</sub>/min/kg ( $3.36 \pm 0.54$  l/min) peak oxygen uptake, and  $690 \pm 23$  Newton meters (Nm) knee peak isometric extension strength) who gave informed consent and passed a comprehensive medical examination and treadmill test. On the basis of age, peak  $\dot{V}O_2$ , and leg extension strength, the subjects were placed into three comparable groups: ITE training (N = 7), IKE training (N = 7), and NOE training (N = 5) (ref. 12). Peak  $\dot{V}O_2$  for the ITE, IKE, and NOE groups was  $3.46 \pm \text{SD } 0.68$  l/min,  $3.38 \pm 0.36$  l/min, and  $3.19 \pm 0.58$  l/min, respectively. Leg total strengths were  $714 \pm \text{SD } 112$  Nm,  $704 \pm 102$  Nm, and  $645 \pm 86$  Nm, respectively.

## Protocol

There were two 41.5-day experimental periods (7 days ambulatory control, 30 days of 6-deg HDBR, and 4.5 days of ambulatory recovery) conducted after a 3-mo ambulatory familiarization period (ref. 12). Twelve men (4 ITE, 4 IKE, and 4 NOE) were tested in July and August 1986; one week later, the other 7 subjects (3 ITE, 3 IKE, 1 NOE) were tested in August and September. The horizontal or head-down positions were maintained

throughout bed rest including showering and excretory functions; they were allowed one pillow and could rise on one elbow to eat. Mean ( $N = 19$ ) caloric consumption was  $2,813 \pm SE 47$  kcal/d and fluids were consumed ad libitum (ref. 15).

### Exercise Training and Testing

Peak oxygen uptake and peak isokinetic strength and endurance were measured before the experiment and then weekly during HDBR in the three test groups.

ITE training required 2-min work periods at 40%  $\dot{V}O_2$  peak alternated with 2-min each at 60%, 70%, 80%, 90%, and 80%  $\dot{V}O_2$  peak. IKE training consisted of 10 bouts of 5 repetitions/10 sec of peak knee flexion and extension force (100-deg range of motion) at a speed of 100 deg/sec followed by 50 sec rest, for a total time of 15 min with each leg, including 2.5-min warm-up and cool-down periods (ref. 13).

### Magnetic Resonance Imaging (MRI)

On control day minus 3 ( $C - 3$ ) and recovery day 3 ( $R + 3$ ) the subjects were transported in a reclining position to the University of California, San Francisco, Radiology Imaging Laboratory where they were tested in the supine position. Their legs were scanned in a quadrature detection head coil that gave an in-plane resolution of  $0.95 \times 0.95$  mm and a 1.0 cm slice thickness. Since the imager had only a 20 cm field of view, the leg was imaged sequentially in three 20-cm segments (i.e., 60 cross-sectional image slices at 1-cm intervals starting from the ankle (fig. 1)). The leg was translated 18 cm for each segment of images and the foot was taped to a lucite leg rest marked to provide reproducible positioning. Separations between the centers of four oil-filled lucite tubes in the leg/foot rest indicated changes in imaging conditions. The distances between the centers of the four tubes for the 20 sections in segments 1 and 2 are presented in table 1; error range was 3.74% to -3.17%. The standard testing mode was a spin echo image with 1.0 sec time to recovery and 30 ms time to echo. Each of the 2,280 cross-sectional slices was enlarged and filtered to provide a modified image for computer analysis (fig. 2, left column). The images were then combined to form a three-dimensional shaded surface display where locations of the light source and observer could be varied (fig. 2, right column).

The first step in the analysis utilized an edge-detection program to outline the total area of the leg that contained all structures except subcutaneous fat; tibia and fibula areas were subtracted from the total area. Image quality was not sufficient to delineate boundaries for the soleus and gastrocnemius muscles in most sections, so the combined PLG muscles (lateral and medial gastrocnemius, soleus, flexor digitorum longus, tibialis, and flexor hallucis longus) and their connecting interosseous membranes were measured. The computer edge-detection program was employed, with manual intervention at obscure points, to outline the border and measure the area of PLG for each of the 60 sections. For example, the soleus and gastrocnemius muscles from an NOE subject were traced manually. The image quality of his cross-sectional slices was sufficient to allow delineation of the soleus pre-bed-rest in segment 1 at about 11 cm above the ankle (fig. 2, lower left), the soleus plus the distal heads of the gastrocnemius pre-bed-rest in segment 2 at about 22 cm above the ankle (fig. 2, middle left), and only the proximal two heads of the gastrocnemius pre-bed-rest in segment 3 at about 42 cm above the ankle (fig. 2, upper left). This method for determination of muscle edges is more accurate than using relaxation time and pixel intensity (ref. 16), but is more labor intensive.

### Statistical Analysis

The data were analyzed with the appropriate paired or unpaired t-tests and linear regression. The null hypothesis was rejected when  $p < 0.05$  for the t-tests and  $p < 0.02$  for the linear regressions. Non-significant differences were NS.

### Results

Mean ( $\pm SE$ ) total muscle group volumes decreased ( $p < 0.05$ ) from control levels in all three test groups after bed rest: ITE by  $4.3 \pm 1.6\%$ , IKE by  $7.7 \pm 1.6\%$ , and NOE by  $6.3 \pm 0.8\%$  (table 2, fig. 3). Only one ITE subject had an apparent increase (NS) in his muscle volume. The ranges of volume changes were 2.6 to -9.0% (ITE), -2.1 to -14.9% (IKE), and -3.4 to -8.1% (NOE). There were no significant differences in decreased volumes between groups; -4.3% (ITE) was not different from -7.7% (IKE). Thus, neither ITE nor IKE training influenced the usual level of atrophy of the PLG muscles during HDBR.

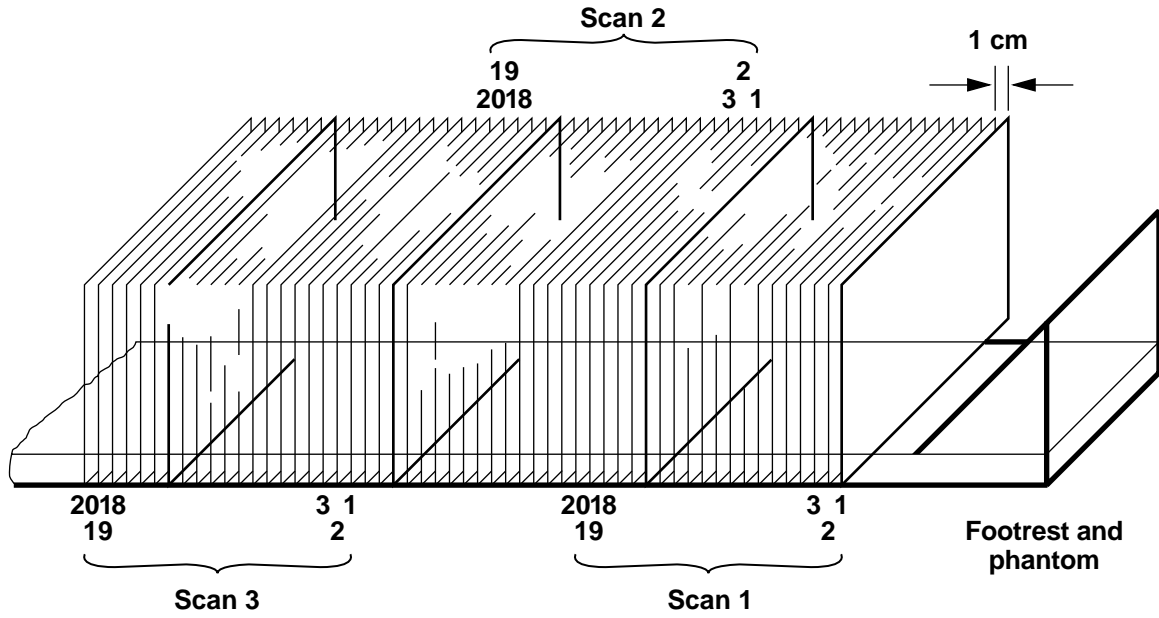


Figure 1. Magnetic resonance imaging sequence.

**Table 1. Mean ( $\pm$  standard error (SE)) distances (pixels) between centers of the four oil-filled calibration tubes in leg segments 1 and 2 in one NOE subject**

	Tubes 1–2	Tubes 2–3	Tubes 3–4	Tubes 4–1
Pre-bed-rest (C – 3)				
Segment 1	93.49 $\pm$ 1.57	63.62 $\pm$ 1.0	52.92 $\pm$ 0.1	209.80 $\pm$ 0.52
Segment 2	74.94 $\pm$ 1.15	79.78 $\pm$ 1.10	52.62 $\pm$ 0.21	207.14 $\pm$ 0.30
Post-bed-rest (R + 3)				
Segment 1	90.52 $\pm$ 1.24	66.00 $\pm$ 1.02	53.04 $\pm$ 0.21	209.42 $\pm$ 0.29
Segment 2	76.89 $\pm$ 1.29	78.53 $\pm$ 1.08	52.63 $\pm$ 0.19	207.94 $\pm$ 0.35
% Segment 1	–3.17	3.74	0.23	–0.18
% Segment 2	2.60	–1.57	0.02	0.37

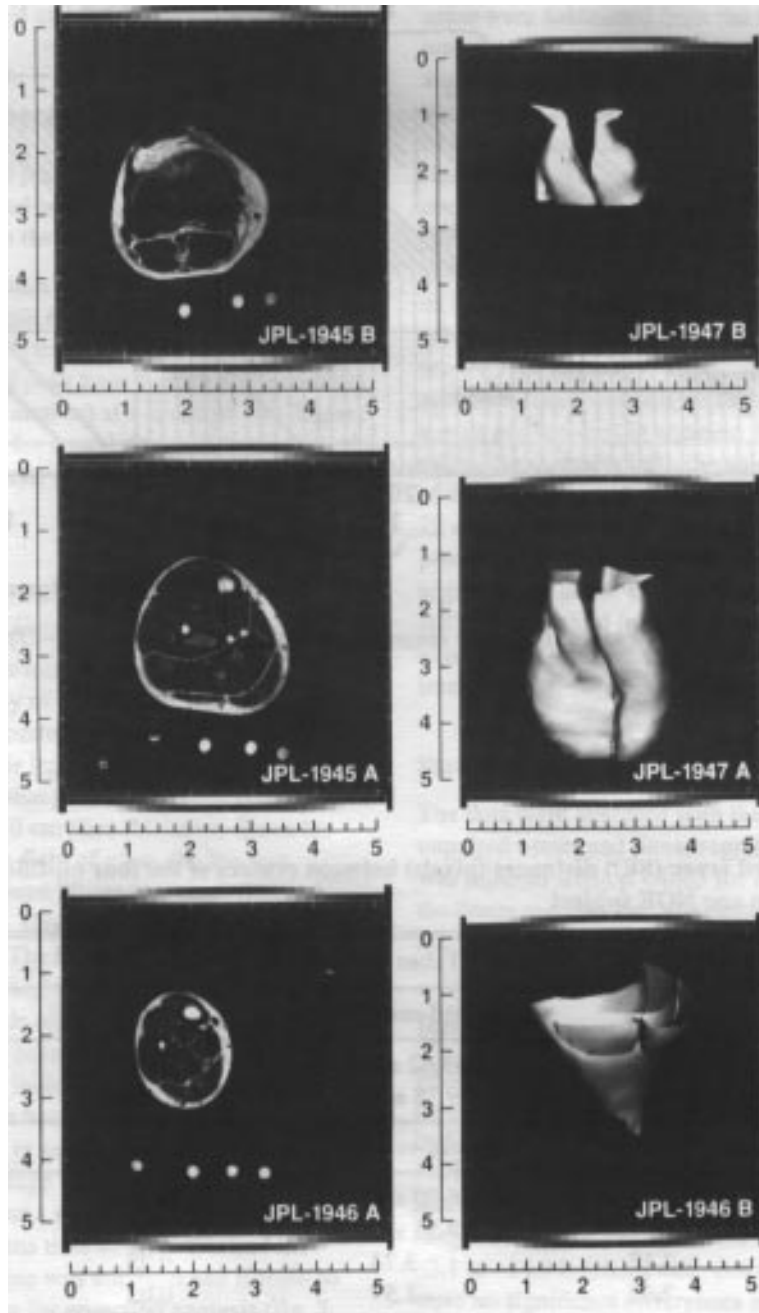
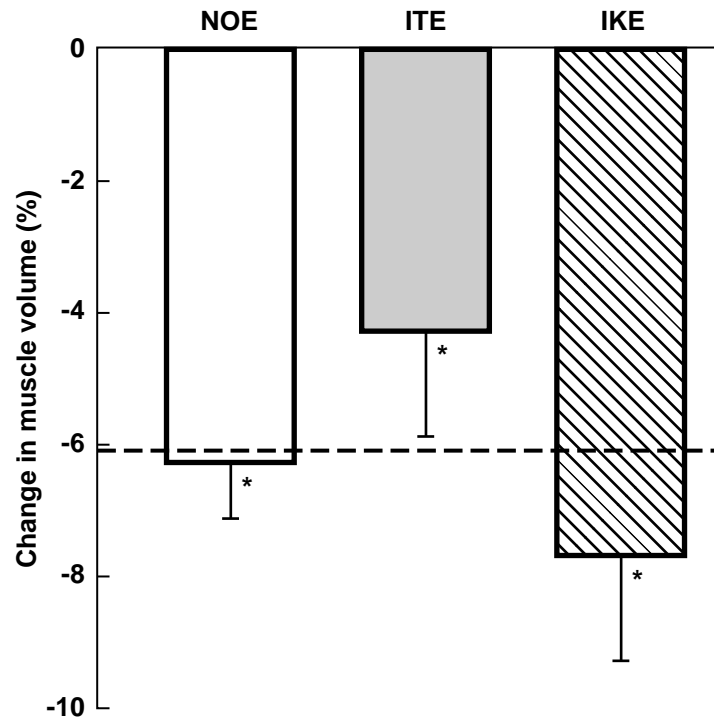


Figure 2. Cross-sectional magnetic resonance images (left column) and posterior surface reconstruction views (right column) of the left leg of test subject. Lower left: pre-HDBR soleus in segment 1 at 11 cm above the ankle; lower right: post-HDBR tendon of the triceps surae insertion. Middle left: pre-HDBR soleus and the medial and lateral heads of the gastrocnemius in segment 2 (22 cm); middle right: pre-HDBR medial and lateral heads of the gastrocnemius (the soleus is hidden). Upper left: pre-HDBR medial and lateral heads of the gastrocnemius in segment 3 (42 cm); upper right: post-HDBR heads of only the gastrocnemius at 42 cm behind the knee. The four circles seen in the left-column images are the oil-filled tubes.

**Table 2. Mean ( $\pm$  SE) volumes (pixels) of the posterior leg muscles in the ambulatory control (C – 3) and recovery (R + 3) periods in the three test groups**

	C – 3	R + 3	% $\Delta$
No exercise (N = 5)			
$\bar{X}$	536,478	502,190	$-6.3^a$
$\pm$ SE	40,156	35,834	0.8
Isotonic exercise (N = 7)			
$\bar{X}$	538,541	516,688	$-4.3^a$
$\pm$ SE	36,942	40,456	1.6
Isokinetic exercise (N = 7)			
$\bar{X}$	574,858	530,892	$-7.7^a$
$\pm$ SE	15,335	17,336	1.6
All subjects (N = 19)			
$\bar{X}$			$-6.1^a$
$\pm$ SE			0.9

<sup>a</sup> $p < 0.05$  from zero.



*Figure 3. Mean ( $\pm$ SE) percent change in PLG muscle volumes (pixels) from ambulatory control (C – 3) to recovery (R + 3) for the three test groups. Dashed line is the mean loss of the three groups. \* $p < 0.05$  from zero.*

## Discussion

The significant decrease in leg-muscle volume by 6.1% in the NOE group was expected and was similar to the reductions in calf muscle area (computer-aided tomography scan) of 4.8% (ref. 3) and leg-triceps volume (MRI) of 10.5% (ref. 2), which were measured after 30 days of 6-deg HDBR. The unexpected finding was that the two exercise training regimens did not attenuate the loss observed in the NOE group. There were tendencies (NS) for lower volume loss (4.3%) with ITE and greater loss (7.7%) with IKE. The subjects, although confined to the head-down position, were relatively active physically with their daily testing, showering, and recreational regimens. Thus, the level of atrophy of the NOE group may have been attenuated, which would have reduced the difference between it and the exercise groups.

Knee flexion/extension exercise mainly involves posterior thigh (hamstring) and anterior thigh (quadriceps) muscular group actions, respectively. Posterior leg group muscles mainly act to plantar-flex the foot at the ankle. Presumably the ITE and IKE regimens did not stress the PLG muscles sufficiently to attenuate bed rest-induced atrophy. There was possibly some plantar flexion with ITE training on the cycle ergometer, which may have contributed to the somewhat lower volume loss, but the ankle was essentially fixed with a strap on the isokinetic ergometer arm and that may have caused the greater loss of muscle volume.

The thickness of smaller PLG muscles (soleus, flexor hallucis longus, tibialis posterior) was also measured with ultrasonography on days C – 6 and R + 4 (ref. 14). Regressions of percent change in posterior leg muscle

group thickness (ultrasonic) on percent change in similar posterior leg muscle group volume (MRI) for the three test groups are presented in figure 4. The regression line slopes of the two exercise groups are positive, but the slope for the NOE group is negative. Mean ( $\pm$ SE) percent changes and correlation coefficients in muscle volumes and thicknesses, respectively, were: ITE =  $-4.3 \pm 1.6$  vs.  $-5.7 \pm 2.2\%$  ( $r = 0.79$ ,  $p < 0.05$ ); IKE =  $-7.7 \pm 1.6$  vs.  $-3.6 \pm 1.4\%$  ( $r = 0.27$ , NS); and NOE =  $-6.3 \pm 0.8$  vs.  $-5.1 \pm 1.3\%$  ( $r = 0.63$ , NS). The agreement on changes in muscle group thicknesses and volumes was reasonably good considering that they were measured one day apart in the recovery period, they were measured by different methods, and the muscles measured were not exactly the same.

One factor influencing muscle thickness and volume is water content. To the extent that plasma (extracellular) volume reflects skeletal muscle water content, resting plasma volume levels at the end of HDBR were  $-1.5 \pm 2.3\%$  (ITE, NS),  $-16.8 \pm 2.9\%$  (IKE,  $p < 0.05$ ), and  $-14.7 \pm 2.8\%$  (NOE,  $p < 0.05$ ) (ref. 15). When plasma volume was unchanged during HDBR with ITE, there was a significant correlation ( $r = 0.79$ ,  $p < 0.05$ ) between MRI volume and ultrasonic thickness. When plasma volumes were decreased significantly during HDBR with IKE and NOE, the correlations between muscle volume and thickness ( $r = 0.27$  and  $r = 0.63$ , respectively) were not significant (fig. 4). Thus, these data suggest anatomical leg muscle volume and thickness responses are similar when body hydration (plasma volume) is maintained during bed rest. This positive relationship disappears in hypohydrated-hypovolemic subjects.



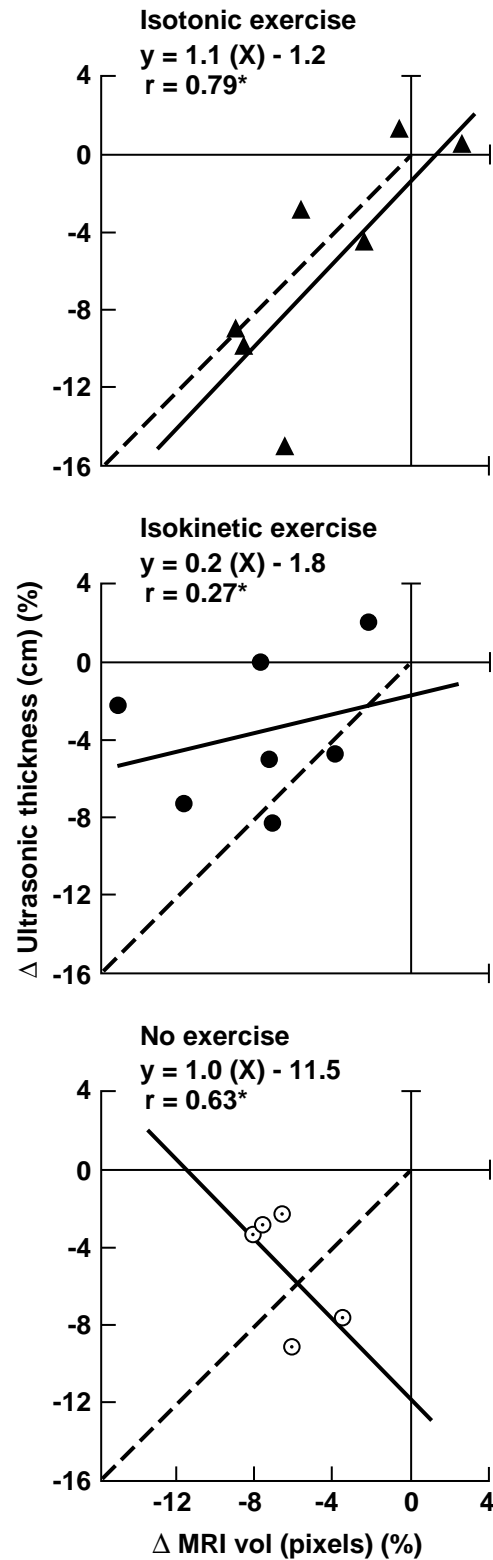


Figure 4. Regression of percent changes (C – 6 to R + 4) in posterior leg muscle group thicknesses (ultrasonography) on percent changes (C – 3 to R + 3) in posterior leg muscle group volumes (MRI) for the three test groups. Dashed line is line of identity.  $p^* < 0.05$ .

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